CLAIMS

We claim:

1. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

a) providing a first probe comprising:

i) an upstream universal priming site (UUP);

ii) an adapter sequence;

iii) a first target-specific sequence comprising a first base at a readout position; and

iv) a downstream universal priming site (DUP);

b) contacting said first probe with said target sequence under conditions whereby only if said first base is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

c) removing non-hybridized first probes;

d) denaturing said hybridization complex;

e) amplifying said first probe to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

g) determining the nucleotide at said detection position.

2. A method according to claim 1 wherein said amplicons comprise a label.

3. A method according to claim 1 further comprising:

a) providing a second probe comprising:

i) an upstream universal priming site (UUP);

ii) an adapter sequence;

iii) a second target-specific sequence comprising a second base at said readout position; and

iv) a downstream universal priming site (DUP);

b) contacting said second probe with said target sequence under conditions whereby only if said second base is perfectly complementary to a nucleotide at said detection position is a second hybridization complex formed;

c) removing non-hybridized second probes;

d) denaturing said second hybridization complex;

e) amplifying said second probe to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

g) determining the nucleotide at said detection position.

4. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

a) providing a plurality of readout probes each comprising:

i) an upstream universal priming site (UUP);

ii) an adapter sequence;

iii) a target-specific sequence comprising a unique base at a readout position; and

iv) a downstream universal priming site (DUP);

b) contacting said detection probes with said target sequence under conditions whereby only if said base at said readout position is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

c) removing non-hybridized first probes;

d) denaturing said first hybridization complex;

e) amplifying said detection probes to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

g) determining the nucleotide at said detection position.

5. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence; and

b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

Ii) a second target-specific sequence comprising a first base at an interrogation position;

wherein if said first base is perfectly complementary to said nucleotide at said

detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

c) removing non-hybridized first probes;

d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

e) amplifying said ligated probe to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

g) determining the nucleotide at said detection position.

6. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence; and

b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

Ii) a second target-specific sequence comprising a first base at an interrogation position;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

c) removing non-hybridized first probes;

d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

e) hybridizing said ligated probe to a rolling circle (RC) sequence comprising:

i) an upstream priming sequence; and

ii) a downstream priming sequence;

f) providing a ligase that ligates said upstream and downstream priming sites to form a circular ligated probe;

g) amplifying said circular ligated probe to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

g) determining the nucleotide at said detection position.

7. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) hybridizing a rolling circle (RC) probe to said target sequence, said RC probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence;

iii) a second target-specific sequence comprising a first base at an interrogation position; and

iv) an adapter sequence;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed;

c) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

d) amplifying said ligated probe to generate a plurality of amplicons;

e) contacting said amplicons with an array of capture probes; and

f) determining the nucleotide at said detection position.

8. A method according to claim 7, further comprising removing non-hybridized RC probe.

9. A method according to claim 1, 4, 5, 6 or 8 wherein said removing comprises:

a) enzymatically adding a binding ligand to said target sequence;

b) binding a hybridization complex comprising said target sequence comprising said binding ligand to a binding partner immobilized on a solid support;

c) washing away unhybridized probes; and

d) eluting said probe off said solid support.

10. A method according to claim 1, 4, 5, 6 or 8 wherein said removing is done using a double-stranded specific moiety.

11. A method according to claim 10 wherein said double-stranded specific moiety is an intercalator attached to a support.

12. A method according to claim 9 wherein said support is a bead.

13. A method according to claim 1, 4, 5, 6 or 7 wherein said amplifying is done by:
a) hybridizing a first universal primer to said UUP;
b) providing a polymerase and dNTPs such that said first universal primer is extended;
c) hybridizing a second universal primer to said DUP;
d) providing a polymerase and dNTPs such that said second universal primer is extended; and
e) repeating steps a) through d).

14. A method according to claim 1, 4, 5, 6 or 7 wherein said array comprises:
a) a substrate with a patterned surface comprising discrete sites; and
b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

15. A method according to claim 14 wherein said discrete sites comprise wells.

16. A method according to claim 14 or 15 wherein said substrate comprises a fiber optic bundle.

17. A method of determining the identification of a nucleotide at a detection position in a genomic target sequence comprising:
a) attaching a library of genomic target sequences to a solid support;
b) adding at least one probe and an enzyme to form an extended primer;
c) denaturing said extended primer from said target sequence;
d) hybridizing said extended primer to an array comprising capture probes; and
e) determining said nucleotide at said detection position.

18. A method according to claim 17, further comprising removing unhybridized probes.

19. A method according to claim 1, 4, 5, 6 or 7, further comprising providing a support on which the target sequence is immobilized.

20. A method according to claim 19, wherein said non-hybridized first probes are removed without

removing said target sequence from said support.

21. A method according to claim 1, 4, 5, 6 or 7, further comprising attaching said target sequence to a support.

22. A method according to claim 21, wherein said target sequence is attached to said support by a method selected from the group consisting of labeling said target sequence with a functional attachment moiety, absorption of said target sequence on a charged support, direct chemical attachment of said target sequence to said support and photocrosslinking said target sequence to said support.

23. A method according to claim 1, 4, 5, 6 or 7, wherein said support is selected from the group consisting of paper, plastic and tubes.

24. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

a) providing a support on which the target sequence is immobilized;

b) providing a first probe comprising:

i) an upstream universal priming site (UUP);

ii) an adapter sequence;

iii) a first target-specific sequence comprising a first base at a readout position; and

iv) a downstream universal priming site (DUP);

c) contacting said first probe with said target sequence under conditions whereby only if said first base is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

d) removing non-hybridized first probes;

e) denaturing said hybridization complex;

f) amplifying said first probe to generate a plurality of amplicons;

g) contacting said amplicons with an array of capture probes; and

h) determining the nucleotide at said detection position

25. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

a) providing a support on which the target sequence is immobilized;

b) providing a plurality of readout probes each comprising:

i) an upstream universal priming site (UUP);

ii) an adapter sequence;

iii) a target-specific sequence comprising a unique base at a readout position; and

iv) a downstream universal priming site (DUP);

c) contacting said detection probes with said target sequence under conditions whereby only if said base at said readout position is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

d) removing non-hybridized first probes;

e) denaturing said first hybridization complex;

f) amplifying said detection probes to generate a plurality of amplicons;

g) contacting said amplicons with an array of capture probes; and

h) determining the nucleotide at said detection position.

26. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the target sequence is immobilized;

b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence; and

c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

Ii) a second target-specific sequence comprising a first base at an interrogation position;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

d) removing non-hybridized first probes;

e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

f) amplifying said ligated probe to generate a plurality of amplicons;

g) contacting said amplicons with an array of capture probes; and

h) determining the nucleotide at said detection position.

27. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the target sequence is immobilized;

b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence; and

c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

i) a downstream universal priming site (DUP); and

Ii) a second target-specific sequence comprising a first base at an interrogation position;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

d) removing non-hybridized first probes;

e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

f) hybridizing said ligated probe to a rolling circle (RC) sequence comprising:

i) an upstream priming sequence; and

ii) a downstream priming sequence;

g) providing a ligase that ligates said upstream and downstream priming sites to form a circular ligated probe;

h) amplifying said circular ligated probe to generate a plurality of amplicons;

i) contacting said amplicons with an array of capture probes; and

j) determining the nucleotide at said detection position.

28. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the target sequence is immobilized;

b) hybridizing a rolling circle (RC) probe to said target sequence, said RC probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence;

iii) a second target-specific sequence comprising a first base at an interrogation position; and

iv) an adapter sequence;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed;

c) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

d) amplifying said ligated probe to generate a plurality of amplicons;

e) contacting said amplicons with an array of capture probes; and

f) determining the nucleotide at said detection position.

29. A method according to claim 28, further comprising removing unhybridized RC probe.